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Numerical Simulation of Pile-Up Distorted Time-Correlated Single Photon Counting (TCSPC) Data

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A model, which is based on the binominal distribution, is derived for understanding and investigating the effect of statistical pulse pile-up. The model is applied to constant and exponential decaying sources and is compared with some experimental results.

KEY WORDS: Time-correlated single-photon counting (TCSPC); synthetic data; pulse pile-up; binomial distribution.

INTRODUCTION

The TCSPC method is based on the principle that the deactivation probability of an excited molecule via fluorescence at a time t_i after delta pulse excitation is proportional to the fluorescence intensity at that time. When using a time-to-amplitude converter and a multichannel analyzer (MCA) for detection, the true probability distribution of the decay is obtained, if the recorded photon is always the only one reaching the photocathode until the next excitation cycle. In any multiphoton event, only the time information of the first arriving photon is recorded and stored in the MCA, which leads to a statistical distortion of the decay histogram. This so-called pulse pile-up effect or type-S pile-up [1] is due to the nature of the method and can be reduced by limiting the start/stop ratio to 1–2% but cannot be eliminated totally. This has, however, the disadvantage of long data collection times, which can cause the problem of introducing systematic errors [2,3].

The precision of kinetic parameters, which are extracted from the raw data, critically depends on the experimental conditions. Of course it is always desirable to obtain a high time resolution and a high number of counts in the peak channel [4,5]. In contrast, the absolute time of data collection should be minimized, because a possible long time drift of the excitation pulse profile could affect the quality of the data, and in reality there will always be a compromise between statistic precision and measuring time. The optimization of decay experiments is especially important if flashlamps with relatively low repetition rates are used and presumes a detailed understanding about the nature of statistical pulse pile-up and the influence on decay curves, which are discussed in the following. Other effects such as detector dead times (type E pile-up) [1] or cross-channel pile-up due to statistical multiplexing [6,7] are of minor importance for standard TCSPC experiments and are not concerned here.

Various methods for the correction of pulse pile-up are known in the literature [8,9]. Coates [10] as well as Davis and King [11] used an equation to correct the raw data. Analytical functions, which are derived from Poisson statistics, are also known for constant, single- and double-exponential functions and can be used to fit the distorted data directly [1,12,13]. These functions have, however, the disadvantage of being time-consuming in

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deconvolution procedures. Electronic methods for pile-up discrimination have also been discussed [1,14,15].

The statistical nature of TCSPC data is well defined and therefore it is easy to simulate a desired decay. Such simulation studies are now widely used for testing the possibilities and limitations of decay curve analysis approaches [16–19]. Hence, a simple model for the simulation of pile-up distorted decay curves is derived and applied to constant, single- and double-exponential sources. For the case of a convoluted single-exponential decay, the simulation results are compared with experiments using 9-aminoacridine as fluorophore.

MATERIALS AND METHODS

9-Aminoacridine (9AC) (Sigma Chemical Co.) was used as received. The solvent ethanol (Merck) was of spectroscopic grade, purified by distillation, and dried subsequently to remove traces of water. For deoxygenation, the samples were bubbled for 10 min with high-purity nitrogen and then sealed in 1 × 1-cm cuvettes. The excitation wavelength for all measurements was λ_{ex} = 381 nm. Fluorescence emission was detected at λ_{em} = 470 nm. The optical density of the fluorophore at the excitation wavelength was about 0.3.

Fluorescence decay functions were measured employing the technique of time-correlated single-photon counting as described elsewhere [20]. To take into account a possible long time drift in the excitation pulse profile, lamp and fluorescence data were collected alternately. All measurements were carried out at 293 ± 1 K. The counts were collected into a 512-channel segment of the multichannel analyzer with a time resolution of 3.4 Ch/ns. The total number of counts in the peak channel (CPC) was about $5 \cdot 10^3$.

The simulations were carried out on an IBM-compatible personal computer with a 33-MHz 80386 CPU and 80387 coprocessor. The programs are written in GFA-Basic 3.0 (compiler version). The random number generator used was also taken from GFA-Basic 3.0. The randomness was verified by performing different statistical tests (e.g., χ^2, runs up/down, serial) [21,22] and the generator was found to be satisfactory for all required applications.

The model functions $F(t)$ were of an exponential form with n = 1,2.

$$F(t) = \sum_{i=1}^{n} A_i \exp\left(\frac{-t}{\tau_i}\right) \qquad (1)$$

Due to the fact that the instrument response function $L(t)$ is not negligibly short compared to the fluorescence function, realistic decay profiles were obtained from a convolution of $F(t)$ with a synthetic excitation pulse. Here, Eq. (2) was used, giving a pulse shape typical for a hydrogen-filled flashlamp (see Fig. 2).

$$L(t) = \alpha_1 t^2 \exp(-\beta_1(t + b_1)) + \alpha_2[\exp(-\beta_2(t + b_2)) - \exp(-\beta_3(t + b_2))] \qquad (2)$$

Since Poisson statistics is generally assumed for TCSPC data, noise was added according to the algorithm of Box–Muller–Marsaglia [21].

SIMULATION OF STATISTICAL PULSE PILE-UP

The pile-up model to be derived here is based on the binomial distribution and calculates the probability function of pile-up distorted data from an arbitrary decay curve using three assumptions.

(i) A number of n photons is emitted in constant time intervals Δt after an arbitrary excitation pulse. All photons can be distinguished concerning the time of emission (N_1 is the first, N_n is the last).

(ii) The photons can move only in discrete paths. The number of those paths is given by the parameter b.

(iii) From all paths b, only a single one is detected. If more than one photon moves in that detection path, only the first one is recorded.

The analogy of items i–iii to the monophoton experiment is as follows: the photocathode area represents a spherical segment around the excited sample. Only a photon which is emitted toward the spacial direction of the photocathode has a chance of being recorded. It is now easy to imagine that a sphere around the sample is covered with photocathodes (= paths b), but only one of them detects the photons. Moreover, every emitted photon N_i represents a defined time t_i, which is analogeous to a specific channel in the MCA, and thus the counts in path i are incremented if the event N_i occurs.

For a source with a constant pulse rate, all photons are emitted with equal probability after delta pulse excitation. Then q is the probability for a single photon of being emitted via the detection path and is given by $q = 1/b$. The probability $p(k)$ to find a number of k photons in the detection path is defined by the binominal

distribution.

$$p(k) = \binom{n}{k} q^k (1 - q)^{n-k} \tag{3}$$

For the case that $n \gg q$ (as common for monophoton experiments), Eq. (3) can be approximated by the Poisson distribution.

$$p(k) = \frac{(nq)^k}{k!} \exp(-nq) \tag{4}$$

The chance of emitting no photon in the detection path is $p(0) = (1 - q)^n$ and the start/stop ratio can be defined by $1 - p(0)$. For a constant source the intensity profile must be a parallel to the time axis. However, if multiphoton events occur, only the first arriving photon (here the photon with the lowest number) is recorded and thus statistically favored. It is therefore important to decrease the detection probability, which can be achieved by an increasing b. Now the question arises, which probability function results for a constant source with n emitted photons and b paths under the conditions of a monophoton experiment.

Example. Four different photons N_i have five paths. Then $q = 0.2$ and the detection hierarchy is $N_1 > N_2 > N_3 > N_4$. For combining k of four photons $\sum_{k=0}^{4} \binom{4}{k} = 16$ possibilities exist and all events with N_1 are counted for N_1, all events with N_2 but not N_1 are counted for N_2, and so on. The corrected probability q_c of detecting a photon N_i is then

$$q_c(N_1) = 1q(1 - q)^3 + 3q^2(1 - q)^2 + 3q^3(1 - q) + 1q^4 \tag{5}$$

$$q_c(N_2) = 1q(1 - q)^3 + 2q^2(1 - q)^2 + 1q^3(1 - q) \tag{6}$$

$$q_c(N_3) = 1q(1 - q)^3 + 1q^2(1 - q)^2 \tag{7}$$

$$q_c(N_4) = 1q(1 - q)^3 \tag{8}$$

where the factors follow again a binominal distribution. If, for example, two of the four photons are combined, $\binom{4}{2} = 6$ possibilities occur. The detection events are $N_1 = 3$, $N_2 = 2$, $N_3 = 1$, and $N_4 = 0$. From Eqs. (5)–(8) the corrected probabilities $q_c(N_1) = 0.2$, $q_c(N_2) = 0.16$, $q_c(N_3) = 0.128$, and $q_c(N_4) = 0.1024$ are derived, leading to a total count rate of 0.5904.

For the general case with k of n photons, $\binom{n}{k}$ combinations are possible. The chance of detecting a special photon N_i is $\binom{n-i}{k-1}$, where $i \geq n - k + 1$. The total detection probability distribution $\sum_{i=1}^{n} q_c(N_i)$ of a pile-up distorted data set with the channels $i = 1 - n$ is then obtained from the true distribution $q(N_i)$ by the following equation:

$$\sum_{i=1}^{n} q_c(N_i) = \sum_{i=1}^{n} \left[\sum_{k=1}^{n+1-i} \left[\frac{\binom{n-i}{k-1} p(k)}{\binom{n}{k}} \right] \right] \tag{9}$$

Equation (9) can be solved quickly for a constant pulse ($q(N_i) = q(N_{i+1})$) with a fast computer algorithm. The parameters q and b can in this case be calculated from $p(0) = (1 - q)^n$ and from $q = 1/b$. Some results are presented in Fig. 1 for start/stop ratios from 1 to 30% with $n = 250$ photons (= channels in the MCA). When keeping in mind that only the relative probabilities are of importance, each data set can be normalized to the first channel for a better comparison. The influence of pulse pile-up is clearly visible and the resulting curves can be adequately fitted by a single-exponential function as predicted by Holzapfel [12].

Although a constant source can be interesting for testing the linearity of the detection electronics [23], the analysis of decaying curves is of course much more important for scientific applications. In this case where $q(N_i) \neq q(N_{i+1})$, Eq. (9) becomes time-consuming and can hardly be solved in an adequate time for high n. However, the calculation can be truncated after taking into account events up to five photons and it was found that the approximation is nearly undistinguishable compared to the exact solution. Equation (9) has, furthermore, the

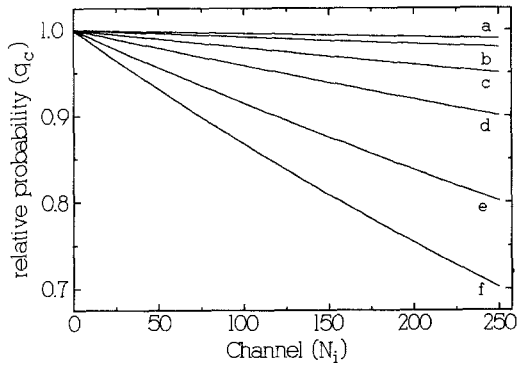

Fig. 1. Influence of statistical pulse pile-up on a constant source with start/stop ratios of 1% (a), 2% (b), 5% (c), 10% (d), 20% (e), and 30% (f). For comparison, each data set is normalized to the first channel (see text).

Table I. Influence of Statistical Pulse Pile-Up on the Single-Exponential Fluorescence Decay of 9-Aminoacridine[a]

Experimental data						Numerical simulation		
TNC	CPC	Stop events (1/s)	Start/stop (%)	τ (Ch)	χ^2	Start/stop (%)	τ (Ch)	χ^2
328031	5084	280	0.84	52.70	1.10	1.00	52.48	0.98
315536	5025	840	2.55	51.69	1.21	2.50	51.89	1.04
319268	5158	2025	6.14	51.12	1.39	6.00	51.76	1.18
318322	5073	3009	9.11	51.05	1.49	9.00	51.50	1.25
324921	5292	4069	12.33	50.82	1.61	12.00	51.01	1.35
321281	5299	5275	15.98	50.12	1.88	15.00	50.36	1.47
313985	5245	6048	18.32	49.72	1.94	18.00	49.76	1.59

[a] Comparison of experimental and simulated data. The probability function used in Eq. (9) was created by a convolution of a single-exponential decay (τ = 52.70 Ch) with an artificial lamp pulse (see Fig. 2) and normalization to $5 \cdot 10^3$ CPC.

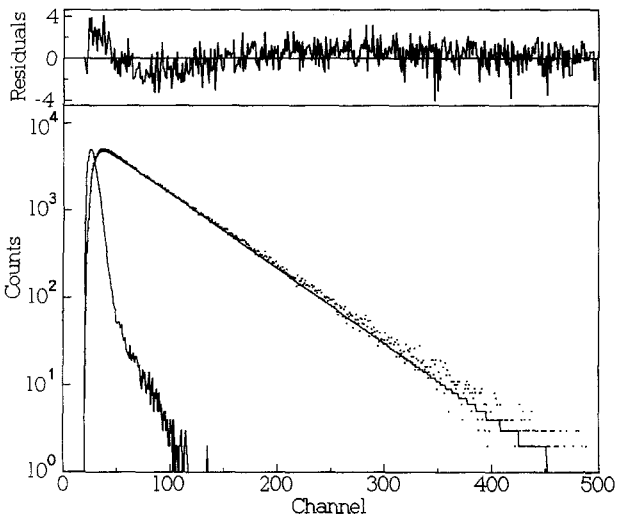

Fig. 2. Simulation of a pile-up distorted fluorescence decay function with τ = 52.70 Ch and a start/stop ratio of 18%, fitted to a single-exponential function. The resulting lifetime was τ = 49.76 Ch and χ^2 = 1.59. The synthetic excitation pulse was created with Eq. (2) and normalized to $5 \cdot 10^3$ CPC. Parameters: $\alpha_1 = 10^4$, α_2 = 500, β_1 = 0.36 Ch, β_2 = 15 Ch, β_3 = 10 Ch, b_1 = 20 Ch, b_2 = 35 Ch.

advantage that arbitrary (especially convoluted) decay functions can be investigated under experimental conditions. This is now demonstrated for the truly single-exponential decay of 9AC in the solvent ethanol.

9AC was chosen because it possesses a high fluorescence quantum yield and a long lifetime of τ = 52.70 Ch (15.5 ns).[3] The experiments were performed with

[3] The true lifetime τ(298 K) = 15.5 ± 0.1 ns for a degassed sample of 9AC in the solvent ethanol was determined with a start/stop ratio of 0.2% (χ^2 = 1.01).

Table II. Influence of Statistical Pulse Pile-Up on a Simulated Double-Exponential Fluorescence Decay with the Model Parameters τ_1 = 75 Ch, τ_2 = 25 Ch, A_1/A_2 = 1.00, and $1 \cdot 10^4$ CPC.

TNC	Start/stop (%)	τ_1 (Ch)	τ_2 (Ch)	A_1/A_2	χ^2
685568	1.00	74.83	24.95	0.984	1.00
678281	2.50	74.53	24.60	0.981	1.03
667917	6.00	74.20	24.52	0.938	1.09
657356	9.00	74.24	24.48	0.900	0.98
645448	12.00	74.60	24.56	0.824	0.97
636921	15.00	74.04	24.12	0.809	1.06
625983	18.00	73.36	23.76	0.785	1.04

start/stop ratios between 1 and 18% with ≈$5 \cdot 10^3$ CPC and ≈$3 \cdot 10^5$ TNC (total number of counts); the results are presented in Table I. A typical single-exponential fit of a pile-up distorted decay is shown in Fig. 2.

As expected, τ decreases with increasing detection probability. For the simulations, the true decay function $F(t) = A \exp(-t/52.7 \text{ Ch})$ was convoluted with an artificial pulse profile and the resulting function $q(N_i)$ was normalized to $5 \cdot 10^3$ CPC. The pile-up distorted functions $q_c(N_i)$ were then calculated with Eq. (9) and it is evident from Table I that the results of simulation are in very good agreement with the experimental data. Analogeous results were obtained from experiments with the fluorophore 1,6-diphenyl-1,3,5-hexatriene (DPH) instead of 9AC [24]. From the resulting lifetimes and χ^2 values, it can also be concluded that the commonly practiced rule of thumb of 1% start/stop ratio is too hard in this case and that acceptable fits are obtained up to 3%. One should, however, keep in mind that this result can-

not be generalized, because the influence of pulse pile-up is strongly dependent on the time resolution and must always be determined for the specific decay.

While the influence of pile-up on a single-exponential function is evident from an increasing χ^2, a more complicated situation arises for a double-exponential decay. To demonstrate this, a simulation with the model parameters $\tau_1 = 75$ Ch, $\tau_2 = 25$ Ch, an amplitude ratio $A_1/A_2 = 1$, and 10^4 CPC (see above for details) was performed. It can be seen from Table II, that an excellent χ^2 is obtained in all cases and the pile-up distortion is not indicated by a bad fit. Only the kinetic parameters, in particular the amplitude ratio A_1/A_2, which decreases drastically, are affected here. Hence, care has to be taken if multiexponential decays are investigated and it could be dangerous to tolerate a start/stop ratio of more than 2% if no further correction methods are applied.

For a standard TCSPC measurement, statistical pulse pile-up will surely not bring up any difficulties; moreover, if powerful excitation sources such as pulsed lasers with high repetition rates or storage ring radiation are used and the experiment is driven in the reversed mode. Difficulties can arise only if low-repetition flashlamps are used and high count numbers (10^6–10^7) are required. This was also pointed out by Birch and Imhof, who stated that the pile-up restriction should not cause a problem in most cases. Nevertheless, they have indicated that higher detection rates can especially be helpful for the determination of rotational parameters from anisotropy measurements, where a great number of total counts is always required [25], and here it is helpful to check the tolerable start/stop ratio in advance.

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